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A method for the immobilization of a strand of double-stranded nucleic acid
- double-stranded DNA and RNA immobilized on a solid adsorbent with a
single-stranded oligonucleotide, using a vector plasmid

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PATENT ASSIGNEE: Tepnel-Med. 1995

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ABSTRACT: A method of immobilizing a strand of double-stranded nucleic acid sample molecule on a solid adsorbent provided with a ss oligonucleotide involves: (1) forming a construct by ligating to the sample molecule (or fragment), a ds nucleic acid binding molecule having a binding sequence which will hybridize to the oligonucleotide; (2) denaturing the construct to produce single-stranded nucleic acid; and (3) hybridizing the binding sequence of the one of the single strands to the oligonucleotide. Kits are also provided. Preferably, the double-stranded nucleic acid binding molecule is linear and is composed of 2 binding sequences separated by at least one restriction site. The binding sequence is separated from a short tail portion of the molecule by at least one restriction site. The binding sequence may be provided in a vector, e.g. a plasmid. The sequence of the target ds nucleic acid sample need not necessarily be known. The target double-stranded nucleic acid sample is effectively tagged with a known sequence which will hybridize to the oligonucleotide immobilized on the solid adsorbent. (16pp)

DESCRIPTORS: DNA probe, RNA %probe% %hybridization%, %double%-stranded%
DNA on solid adsorbent, vector plasmid (Vol.14, No.14)

7205024 93072869

Quantitative and qualitative analysis of amplified DNA sequences by a competitive hybridization assay.

Terouanne B; Balaguer P; Boussioux AM; Nicolas JC

INSERM Unite 58, Montpellier, France.

Anal Biochem (UNITED STATES) Sep 1992, 205 (2) p193-9, ISSN 0003-2697
Journal Code: 4NK

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 9302

Subfile: INDEX MEDICUS

The presence of allelic sequence variations in DNA fragments can be easily detected by measuring the extent of DNA strand exchange between test double-stranded PCR products (target) and labeled standard %double%-stranded PCR products (%probe%). Under selected %hybridization% conditions, sequences identical to the probe decreased the formation of double-labeled hybrid, whereas differing sequences were not efficient enough to compete with the regeneration of the %probe%. A %single% base substitution in the target DNA increased the percentage of remaining double-labeled probe. A general procedure involving denaturation and %hybridization% in solution under different temperature conditions or using different probes enabled sequence identification. The degree of regeneration of double-labeled probe was determined using a bioluminescent assay. We evaluated the specificity of this method with two probes (108 and 131 bp) and several targets with different base substitutions.

Descriptors: *DNA--Analysis--AN; *Nucleic Acid Hybridization; Base Sequence; Methods; Molecular Sequence Data; Polymerase Chain Reaction

CAS Registry No.: 9007-49-2 (DNA)

4289859 85020631

A method for recovering strand-specific probes from nick-translated DNA fragments.

Dutton FL; Chovnick A

Anal Biochem (UNITED STATES) Jul 1984, 140 (1) p121-8, ISSN 0003-2697
Journal Code: 4NK

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Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8501

Subfile: INDEX MEDICUS

A method of preparing strand-specific probes for DNA X DNA or DNA X RNA %hybridizations% is described. Double-stranded DNA fragments are first isolated from any recombinant DNA clone containing the desired sequence, and then labeled in vitro by nick-translation (T. Maniatis, A. Jeffrey, and D. G. Kleid (1975) Proc. Natl. Acad. Sci. USA 72, 1184-1188; P. W. J. Rigby, M. Dieckmann, C. Rhodes, and P. Berg (1977) J. Mol. Biol. 113, 237-251). Sequences homologous to the desired strand are captured by annealing the denatured nick-translate to viral strands of an appropriate M13 clone, and recovered by elution of the resulting hybrids from a column of agarose A50M (Bio-Rad). By this method, separate probes with specificity to either strand, as well as the %double%-stranded% %probe%, may conveniently be prepared from a single nick-translation reaction. Probes may be obtained which are homologous either to the full length of the cloned region or to selected portions thereof by selecting appropriate M13 clones for annealing. The probe is recovered as a population of fragments several hundred bases or less in length, which have been found ideal for saturating liquid %hybridizations%, and should be similarly well suited for in situ %hybridizations% to cytological preparations.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *DNA--Isolation and Purification--IP; *Translation, Genetic ; Bacteriophages--Genetics--GE; Base Sequence; Chromatography, Agarose; Cloning, Molecular; Drosophila melanogaster--Genetics--GE; DNA, Recombinant ; DNA, Viral; Nucleic Acid Denaturation; Nucleic Acid Hybridization; Peptide Fragments--Analysis--AN; Xanthine Dehydrogenase--Genetics--GE

CAS Reg

03807664 82262789

The making of strand-specific M13 probes.

Hu N; Messing J

Gene (NETHERLANDS) Mar 1982, 17 (3) p271-7, ISSN 0378-1119

Journal Code: FOP

Languages: ENGLISH

Document type: JOURNAL ARTICLE

JOURNAL ANNOUNCEMENT: 8212

Subfile: INDEX MEDICUS

A novel approach has been developed for the preparation of highly radioactive, strand-specific M13 probes. A universal primer, complementary to the region 5' to the multiple cloning sites of M13mp7, was used to initiate the DNA synthesis of the complementary strand of the M13 sequence downstream from the inserted sequence. The synthesis of the (-) strand, which was labeled with a radioactively labeled precursor, did not proceed to completion so that the inserted sequence was kept single-stranded. Thus, a partially %double%-stranded %probe% that had the specificity of this inserted sequence was obtained. As an example for the application of single-stranded specific %hybridization% probes, an M13mp7 subclone of a zein cDNA clone of maize (A30) was labeled and used in a dot %hybridization% test to select from the hundreds of M13mp7 subclones of the zein genomic clone, Z4, the sequences complementary to the probe. The specificity of the probe was confirmed by dideoxy chain terminator sequencing experiments.

Tags: Support, U.S. Gov't, Non-P.H.S.

Descriptors: *DNA, Recombinant--Analysis--AN; *Genetic Techniques; *Nucleic Acid Hybridization; Base Sequence; DNA, Viral--Analysis--AN; Escherichia coli--Genetics--GE; Plasmids

CAS Registry No.: 0 (DNA, Recombinant); 0 (DNA, Viral); 0 (Plasmids)